Chronic Vascular Pulmonary Dysplasia Associated with Neonatal Hyperoxia Exposure in the Rat

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ABSTRACT. Chronic sequelae of neonatal hyperoxia was studied in male rats exposed to 0.96–1.0 FiO₂ for the first 8 days of life. At 58 days of age functional and morphologic cardiopulmonary changes were compared with controls. Right ventricular systolic pressure was measured percutaneously under anesthesia and was increased in the O₂ group (29.5 mm Hg $\pm$ 3.1 versus 23.2 mm Hg $\pm$ 3.5, $p < 0.001$). Lung and heart weights were similar between groups. Right ventricular weights however were increased in the O₂ group (0.197 g $\pm$ 0.023 versus 0.175 g $\pm$ 0.020, $p < 0.001$). Air pressure-volume curves were similar but in the O₂ rats saline deflation curves were shifted left and maximal fluid lung volumes were greater (14.1 $\pm$ 1.2 versus 12.0 $\pm$ 0.7 ml, $p < 0.001$). Pulmonary arteries were perfused at 100 cm H₂O with a barium-gel mixture and lungs were fixed at 25 cm H₂O with formalin. Microscopic examination of lungs revealed dysplastic changes of alveolar architecture which included irregularly enlarged alveoli and incomplete alveolar septation. Morphometric studies of the lungs showed that the O₂ rats had an increased volume proportion of parenchyma (0.865 $\pm$ 0.020 versus 0.850 $\pm$ 0.019, $p < 0.05$), increased mean linear intercept (72.3 μ m $\pm$ 9.5 versus 53.6 μ m $\pm$ 5.0, $p < 0.001$), decreased number of alveoli per mm² (207 $\pm$ 34 versus 319 $\pm$ 39, $p < 0.001$) and fewer small arteries (20–200 μ m) per mm² (8.7 $\pm$ 1.3 versus 14.9 $\pm$ 2.4, $p < 0.001$). The number of small arteries/100 alveoli was similar. We conclude that limited and specifically timed neonatal hyperoxia is associated with chronic morphologic and functional cardiopulmonary changes in the adult rat. These changes include a decrease in the number of small pulmonary arteries, elevated right ventricular pressure, and emphysema-like changes in the air spaces. (*Pediatr Res* 21: 14–20, 1987)

Abbreviations

RA, room air
P-V, pressure-volume
MLV, maximal lung volume
C_{30–60}, static lung compliance
C_{30–60}/MLV, specific lung compliance
RV, right ventricle
LV + S, left ventricle plus septum
LM, mean linear intercept
ISA, internal surface area
MLV₄₅, MLV at 45 cm H₂O transpulmonary pressure

MLV₁₄, maximal saline lung volume at 14 cm H₂O transpulmonary pressure

Exposure of the lung to increased concentrations of inspired oxygen is associated with multiple toxic effects. These include pulmonary endothelial damage, intraalveolar hemorrhage, development of pulmonary edema, and decreased pulmonary compliance (1–5). The neonate tolerates exposure to hyperoxia with less acute injury than does the adult (6–14). To the neonate, however, hyperoxia presents additional risks by threatening normal growth and development of the lung. In tissue culture, hyperoxia inhibits the growth and extension of pulmonary endothelial tissue (15). *In vivo*, hyperoxia has been associated with decreased DNA synthesis, decreased lung growth, delayed maturation of alveolar structures, and dysplastic pulmonary vascular development (12, 13, 16–20).

The dose and timing dependent relationships of oxygen-induced pulmonary injury are not entirely clear. Long-term sequelae of neonatal hyperoxia exposure have only recently been investigated. Roberts *et al.* (19) exposed newborn rats to hyperoxia for the first 6 days of life and studied pulmonary changes during the following 2 wk. Abnormalities in lung growth and morphology were initially apparent but became attenuated during the observed recovery period. By 2 wk of recovery the lungs were similar to controls in most respects. However, Wilson *et al.* (20) investigated the long-term sequelae of prolonged hyperoxia exposure in the young rat.

Oxygen exposure was begun at 10 days of age and continued for 2–8 wk. Neonatal hyperoxia exposure for as brief a period as 2 wk was found to be associated with impaired alveolar growth and vascular abnormalities in the adult. These investigations suggest therefore that pulmonary growth abnormalities may accompany either acute or prolonged hyperoxia exposure. The results of these studies differ, however, regarding potential for recovery and long-term implications of these pulmonary changes.

The risk of permanent lung damage due to hyperoxia exposure may be related to the timing as well as the duration and concentration of exposure. Burri (21) has reviewed postnatal lung growth in rats and observed a period of tissue proliferation which occurs between 4 and 13 days of postnatal life. It is during this period that alveolar number, surface area, and capillary volume increase through a process of secondary alveolar septal formation. Hyperoxia during this phase of development of the lung may inhibit alveolar septation and thereby cause permanent alterations in morphology and function.

The purpose of this study is to expose neonatal rats to hyperoxia during the first 8 days of life which is similar to the design of Roberts *et al.* (19) followed by a 52-day recovery period similar

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to the design of Wilson *et al.* (20). The chronic effects of this specifically timed and limited neonatal hyperoxia exposure will be assessed both by morphometric and functional cardiopulmonary studies.

MATERIALS AND METHODS

Oxygen exposure. One hundred eight Sprague Dawley newborn rats, 2–18 h of age with their dams, were divided into two groups and received hyperoxia (0.96–1.0 FiO₂) or room air for 8 full days. The animals were housed in regular rat cages which were placed into two 12 ft³ lucite chambers. Each chamber received either humidified O₂ or RA at 10 liter flow per min. Oxygen concentration was measured continually with an oxygen analyzer (Ventronics, Temecula, CA). The CO₂ concentration was measured intermittently and did not exceed 0.2%. Dams were given rat food and water *ad libitum* and were rotated at 24-h intervals between O₂ and RA groups. At the completion of 8 full days, the O₂ group was abruptly returned to room air. The animals were weighed daily for the first 10 days of life, every 2nd day through 11–22 days, and weekly until 60 days of age. Rats were weaned at 22 days of age and were fed rat food until sacrificed at 60 days of age.

In vivo physiologic measurements. At 16 and 90 h following abrupt termination of hyperoxia, arterial oxygen saturations were measured in O₂ and RA groups of rats while they breathed RA. Oxygen saturations were measured over the right carotid artery using a pulse oximeter (Nellcor Inc., Hayward, CA).

At 58 days of age the rats were lightly anesthetized with Halothane and right ventricular systolic and diastolic pressures were measured. A 1-inch 23-gauge needle was inserted percutaneously at the left ziphoid sternal notch and directed transdiaphragmatically into the right heart. A pressure transducer (Millar Instruments, Inc., Houston, TX; model PR249) was placed in the needle hub prior to the puncture. The system was calibrated with a water manometer and zeroed at the level of the right atrium. The pressure wave form was recorded on an Electronics for Medicine 6-channel recorder. Right ventricular pressures were calculated from the mean of 10 consecutive pressure curves.

In 19 O₂ rats and 17 RA rats, right ventricular pressures were repeated twice over a 48-h period. The first measurement was obtained while rats breathed RA mixed with halothane. The second measurement was obtained while rats breathed 85% O₂ mixed with halothane. The purpose of this was to determine if right ventricular systolic pressure, and therefore the peak pulmonary artery pressure in the O₂ rats, was labile and could be decreased by elevating the inspired O₂ concentration.

Preparation of heart and lungs. At 60 days of age, O₂ and RA groups of rats were separated at random into three groups. All animals were anesthetized with 50 mg/kg of sodium pentobarbital injected intraperitoneally.

Rats from the first group were used to obtain lung weights, protein, and DNA content. Animals were sacrificed by exsanguination from the abdominal aorta. Lungs were excised and wet weights recorded. The right lung was frozen for later analysis of DNA and protein content (22–23). The left lung was used for dry weight measurement.

Lungs from the second group were used to obtain P-V curves. The trachea of each rat was cannulated and the animal was denitrogenated by breathing 100% oxygen for 10 min. Lungs were then collapsed by occluding the tracheal cannula. Immediately following cessation of respirations the animals were exsanguinated. The collapsed lungs and heart were excised *en bloc* and a P-V curve was obtained with either air or saline.

Air curves were performed by inflating the collapsed lung during a 30-min period to a maximum pressure of 45 cm H₂O. Volume was read directly from a calibrated syringe and the pressure was measured with a water manometer. Deflation was accomplished over 15 min, with volumes recorded at 5 cm H₂O

pressure decrements to a pressure of 5 cm H₂O; thereafter volumes were recorded at 1 cm H₂O decrements. During deflation, readings were taken only if the pressure remained constant for 1 min. Saline pressure curves were obtained using the method of Mead *et al.* (24). A maximum pressure of 14 cm H₂O was used.

P-V curves were constructed by plotting transpulmonary pressure *versus* volume. Volume was also expressed as a percentage of MLV and as the volume per g of dry lung weight. C_{30–60} was measured as the slope of the straight part of the V-P deflation curve between 30 and 60% of MLV. C_{30–60}/MLV was calculated by dividing the static compliance by the MLV.

The third group of animals was used for morphometric analysis. Each animal was killed by exsanguination. The thorax was widely split and the trachea was cannulated just above the carina. The pulmonary artery was cannulated via the right ventricle with a 13-gauge blunt needle and tied into place. The pulmonary artery was injected at 100 cm H₂O pressure for 4 min with a radioopaque barium gelatin mixture heated to 60° C (25). The heart was excised and the right ventricle was packed with cotton and the entire heart was fixed in 10% buffered formalin. The RV was dissected from the LV + S and each was weighed separately and expressed as the ratio RV/LV + S (26). The lungs were inflated at 25 cm H₂O with 10% buffered formalin and perfused at this pressure for 72 h.

After fixation, lung volume was determined by fluid displacement (27). A calibrated marker was placed on the lung at the level of the pulmonary artery and radiographs were obtained. Pulmonary arteriograms were assessed using a dissecting microscope to measure the left pulmonary artery diameter 5 and 25 mm distal to the main pulmonary artery.

Transverse sections of the superior, middle, and inferior portions of the left lung were processed and embedded in paraffin. The entire transverse sections were cut 5- μ thick and were stained with hematoxylin and eosin or Miller Van Gieson stain.

Volume proportions of the lung nonparenchymal and parenchymal structures were assessed using the point counting system (28). Point counting of nonparenchyma was carried out under low magnification ($\times 35$). The entire area of each section of the left lung was examined and the number of hits on pleura, septa, peribronchial and perivascular tissue, terminal bronchi, and larger bronchi and vessels ($>200 \mu\text{m}$) were recorded. Using a higher magnification ($\times 100$) and covering 20 or more consecutive fields and at least 500 points, the number of parenchymal hits on alveolar air spaces, alveolar ducts, respiratory bronchioles, septal tissue, and vessels $\leq 200 \mu\text{m}$ were counted.

Lm was measured using crossed hair lines of known length. Using $\times 100$ magnification, 20 consecutive fields were counted for each lung (28). The ISA of the lungs was calculated using the mean linear intercept method from the equation: (28)

$$\text{ISA} = \frac{4 \lambda V}{\text{Lm}}$$

where V is the volume of the lung and λ equals the fraction of lung containing parenchyma.

Vessel and alveolar counts were obtained by counting the number of small arteries (25–200 μm external diameter) and alveoli included on a 0.36 mm² grid consisting of 36 squares. A minimum of 20 consecutive fields were counted on each of the three lung sections. At least 1500 alveoli were also counted. From these data the number of alveoli/mm², small arteries/mm², and number of arteries per 100 alveoli were calculated.

Arterial wall medial thickness and extent of muscularization were categorized by vessel external diameters (25–50, 51–100, 101–200, 201–300, 301–400, 401–500, 501–1000, or $>1000 \mu\text{m}$). External diameter was defined as the distance between the outer edges of the external elastic lamina. Medial thickness was defined as the distance along the diameter from the luminal

surface of the internal elastic lamina to the outer edge of the external lamina. The percent medial wall thickness was calculated by (25):

$$\frac{2 \times \text{medial thickness} \times 100}{\text{external diameter}}$$

Muscle tissue was also identified as either circular or oblique (29). Muscle tissue was assessed in 30 consecutive vessels of external diameter 25–50 and 51–100 μm ; and in all arterial vessels 101 to 1000 μm in the three sections from the left lung.

Morphologic data were not corrected for tissue shrinkage due to fixation or processing. The results of each analysis were assessed statistically by unpaired Student's *t* test. Results are expressed as mean values $\pm$ 1 SD.

RESULTS

Growth. All surviving animals appeared healthy at the end of 8 days of hyperoxia and at 60 days of age. However, there was a 3.0% mortality rate in the oxygen group which occurred during the hyperoxia. There were no significant body weight differences between RA and O₂ groups at any age.

In vivo physiologic measurements. Percent oxygen saturation measured in O₂ rats 16 h after abrupt withdrawal from hyperoxia was significantly decreased compared to RA controls ($90\% \pm 3$ versus $97\% \pm 2$, $p < 0.001$). At 90 h of age, however, there was no longer a difference, with oxygen saturation being $96\% \pm 3$ in the O₂ group.

At 58 days of age, systolic right ventricular pressures measured while breathing RA were significantly elevated in the oxygen group ($29.5 \text{ mm Hg} \pm 3.1$) compared to RA controls ($23.2 \text{ mm Hg} \pm 3.5$, $p < 0.001$). Right ventricular pressures in either group were not significantly different when the inspired oxygen concentration was increased to 0.85 (Fig. 1).

Biochemical analysis. There was a small but significant decrease in the total lung DNA in the O₂ group ($4.5 \text{ mg} \pm 0.3$ versus $4.9 \text{ mg} \pm 0.5$, $p < 0.05$) as was the ratio of DNA to dry lung weight (28.2 ± 2.7 versus 32.5 ± 3.6 , $p < 0.001$). A small elevation was observed both in the total lung protein in the O₂ group ($118.5 \text{ mg} \pm 6.5$ versus $113.5 \text{ mg} \pm 6.8$, $p < 0.05$) as was the protein to DNA ratio (16.8 ± 1.2 versus 14.8 ± 2.1 , $p < 0.005$) (Table 1).

Cardiac chamber size. Total heart weight and LV + S weight were similar in the two groups. In the oxygen group, RV weight was significantly greater ($0.197 \text{ g} \pm 0.023$ versus $0.175 \text{ g} \pm 0.020$,

Fig. 1. The chronic effects of neonatal hyperoxia on right ventricular pressure. The hyperoxic animals (dark columns) had a significant increase in pressure compared to RA controls ($p < 0.001$). Ventricular pressure measured while breathing room air or 0.85 O₂ did not alter the results.

$p < 0.001$). The RV/LV + S ratio was also significantly greater among O₂ rats (0.305 ± 0.034 versus 0.271 ± 0.021 , $p < 0.001$).

Lung volume and P-V curves. MLV₄₅, dry lung weights, dry lung weight per g body weight, and MLV per g body weight were similar. The air P-V curves were similar, whether expressed as pressure versus lung volume, percent MLV or lung volume per g dry lung weight. Similarly, static and specific compliances were not different (Table 2).

However, MLV₁₄ was significantly elevated in the O₂ group compared to controls ($14.1 \text{ ml} \pm 1.2$ versus $12.0 \text{ ml} \pm 0.7$, $p < 0.001$). MLV per g of dry lung weight and MLV per g of body weight were significantly larger in the oxygen group (Table 2). Lung dry weight and lung dry weight per g of body weight were not different.

The saline P-V curve expressed as pressure versus percent of MLV was significantly shifted to the left at 6 cm H₂O transpulmonary pressure (Fig. 2). The P-V curves, when expressed as pressure versus volume per g dry lung weight or lung volume were also significantly different (Fig. 3). That is, at any given transpulmonary pressure there was significantly more fluid in the lung. Both the static and the specific compliances were also significantly elevated in the oxygen group (Table 2).

Morphometric studies. The alveolar architecture was disrupted in O₂ rats illustrated by the emphysema-like appearance of the lung. Alveolar septa appear foreshortened and alveoli are enlarged and irregular (Fig. 4). In the oxygen group alveoli were larger in size (mean linear intercept $72.3 \mu\text{m} \pm 9.5$ versus $53.6 \mu\text{m} \pm 5.0$, $p < 0.001$) and the number of alveoli/mm² was

Table 1. Body and lung wt and biochemical measurements in 15 RA and 16 hyperoxic-treated rats

Parameter	RA	O ₂	p
Body wt (g)	276 $\pm$ 11	274 $\pm$ 10	NS
Lung wet wt (g)	1.184 $\pm$ 0.067	1.125 $\pm$ 0.123	NS
Lung dry wt (g)	0.239 $\pm$ 0.019	0.253 $\pm$ 0.024	NS
Total lung DNA (mg)	4.9 $\pm$ 0.5	4.5 $\pm$ 0.3	<0.05
Lung DNA/lung dry wt (mg/g)	32.5 $\pm$ 3.6	28.2 $\pm$ 2.7	<0.001
Total lung protein (mg)	113.5 $\pm$ 6.8	118.5 $\pm$ 6.5	<0.05
Protein/DNA	14.8 $\pm$ 2.1	16.8 $\pm$ 1.2	<0.005

Table 2. P-V measurements and compliances in RA and hyperoxic-treated rats

	RA (n = 14)	Hyperoxic (n = 12)	p
Air P-V data			
Max lung vol (ml)	14.2 $\pm$ 1.3	13.9 $\pm$ 1.1	NS
Lung dry wt/body wt	0.088 $\pm$ 0.007	0.089 $\pm$ 0.006	NS
Max lung vol/body wt (ml/g)	0.051 $\pm$ 0.005	0.050 $\pm$ 0.004	NS
Max lung vol/dry lung wt (ml/g)	58.8 $\pm$ 5.9	56.4 $\pm$ 4.2	NS
C ₃₀₋₆₀ ml/cm H ₂ O	1.372 $\pm$ 0.205	1.376 $\pm$ 0.150	NS
C ₃₀₋₆₀ /MLV	0.097 $\pm$ 0.01	0.098 $\pm$ 0.007	NS
Saline P-V data			
	(n = 9)	(n = 12)	
Max lung vol (ml)	12.0 $\pm$ 0.7	14.1 $\pm$ 1.2	<0.001
Lung dry wt/body wt	0.088 $\pm$ 0.003	0.088 $\pm$ 0.005	NS
Max lung vol/body wt (ml/g)	0.042 $\pm$ 0.003	0.048 $\pm$ 0.003	<0.001
Max lung vol/dry lung wt (ml/g)	48.3 $\pm$ 3.7	55.0 $\pm$ 5.1	<0.002
C ₃₀₋₆₀ ml/cm H ₂ O	1.374 $\pm$ 0.214	1.869 $\pm$ 0.328	<0.002
C ₃₀₋₆₀ /MLV	0.111 $\pm$ 0.011	0.132 $\pm$ 0.013	<0.002

Fig. 2. Static deflation saline-filled P-V curve in room air and oxygen groups. Volume (mean $\pm$ SD) is expressed as percent of volume at 14 cm H₂O (V_{14}). The oxygen group has significantly more volume at 6 cm H₂O transpulmonary pressure ($p < 0.05$).

Fig. 3. Static deflation saline-filled P-V curve in RA and O₂ group. The actual liquid lung volume (ml) is plotted against the transpulmonary pressure. The *asterisks* indicate a significant increase of lung volume (mean $\pm$ SD) in the oxygen group ($p < 0.005$).

Fig. 4. Contrasting light microphotographs of lung parenchyma of RA and O₂ groups. Both microphotographs taken at same magnification, $\times 160$. Note the large alveoli in the O₂ group.

significantly decreased (207 ± 34 versus 319 ± 39 , $p < 0.001$). There was a small but significant increase in the volume proportion of parenchyma in the O_2 group (0.865 ± 0.020 versus 0.850 ± 0.019 , $p < 0.05$) (Table 3).

Parenchymal air space (sum of the space occupied by alveoli, alveolar ducts, and respiratory bronchioles) was also increased in the O_2 group ($64.0\% \pm 5.0$ versus $57.9\% \pm 3.1$, $p < 0.001$). The internal surface area of the lungs was significantly decreased in the O_2 group ($6250 \text{ cm}^2 \pm 720$ versus $8039 \text{ cm}^2 \pm 1225$, $p < 0.001$).

From arteriogram measurements, the external diameter of the left pulmonary artery 5 and 25 mm distal to the main pulmonary artery was significantly smaller in the oxygen group ($1.1 \text{ mm} \pm 0.2$ versus $1.4 \text{ mm} \pm 0.2$, $p < 0.001$; and $0.3 \text{ mm} \pm 0.1$ versus $0.5 \text{ mm} \pm 0.1$, $p < 0.001$).

The number of small arteries (25–200 μm external diameter) per mm^2 was significantly decreased in the O_2 group compared to controls (8.7 ± 1.3 versus 14.4 ± 2.4 , $p < 0.001$). However,

the number of small arteries per 100 alveoli as well as the number of alveoli per arteries were not different (Table 3).

When vessels containing only circular muscle were assessed, there was an increase in the percent medial wall thickness of arteries of all sizes in the hyperoxic animals (Fig. 5). However, the percentage of vessels 101–1000 μm containing oblique muscle was decreased in the O_2 group. In fact, in arteries of the 401–1000 μm size, there was no oblique muscle in the O_2 group, whereas in the RA group 91% of these arteries contained oblique muscle.

In O_2 rats there was distal extension of medial muscle as evidenced by a significant increase in the percentage of small vessels (25–100 μm external diameter) containing circular muscle (Fig. 6).

DISCUSSION

The present investigation demonstrates that hyperoxia exposure limited to the neonatal period is associated with cardiovas-

Table 3. Lung morphometrics in RA and hyperoxic-treated rats

	RA	(n)	O_2	(n)	p
Fraction of lung volume nonparenchyma*	0.150 ± 0.003	(17)	0.135 ± 0.003	(13)	<0.05
Fraction of lung volume parenchyma	0.850 ± 0.019		0.865 ± 0.020		<0.05
Fraction of lung volume airspace†	0.579 ± 0.031		0.640 ± 0.050		<0.001
Fraction of lung volume alveolar septa	0.232 ± 0.024		0.199 ± 0.189		<0.001
Mean linear intercept (μm)	53.6 ± 5.0		72.3 ± 9.5		<0.001
ISA (cm^2)	8039 ± 1225		6250 ± 720		<0.001
No. of small arteries/ mm^2 ‡	14.9 ± 2.4	(18)	8.7 ± 1.3	(15)	<0.001
No. of alveoli/ mm^2	319 ± 39		207 ± 34		<0.001
No. of arteries/100 alveoli	4.5 ± 0.7		4.3 ± 0.8		NS
No. of alveoli/artery	22.7 ± 4.0		24.4 ± 5.9		NS
Diameter of left PA (mm)§		(11)		(10)	
5 mm distal to main PA	1.4 ± 0.2		1.1 ± 0.2		<0.001
25 mm distal to main PA	0.5 ± 0.1		0.3 ± 0.1		<0.001

* Nonparenchyma includes: terminal bronchioles and larger airways, vessels $>200 \mu\text{m}$, and pleura.

† Parenchymal air space includes: respiratory bronchioles, alveolar ducts, and alveoli.

‡ Arteries $\geq 25 \mu\text{m}$ and $\leq 200 \mu\text{m}$.

§ Pulmonary artery.

Fig. 5. Percent medial wall thickness (mean $\pm$ SD) of vessels containing only circular muscle versus external diameter of vessels (μm) in RA and O_2 groups. One and two asterisks indicate $p < 0.05$ and $p < 0.01$, respectively. There were not enough 401 to 1000 μm vessels in RA animals with only circular muscle to derive SDs.

Fig. 6. Percent of pulmonary arterioles (mean $\pm$ 1 SD) 25 to 100 μ m that have medial muscle. The RA animals are identified by the open columns and the O₂ animals by the solid columns. One and two asterisk represent $p < 0.03$ and $p < 0.0002$, respectively.

cular and pulmonary sequelae in the adult rat. We found adult morphologic changes following 8 days of neonatal hyperoxia exposure similar to those reported to occur after more extended exposure in older rats (20). Furthermore, we have extended the use of the rat model to include study of pulmonary and cardiovascular functional changes which accompany these structural changes.

Exposure of the developing lung to increased oxygen concentration has previously been shown to disrupt lung growth (12, 13, 16–18, 20). Decreased long-term lung growth in the present study was suggested by the small but statistically significant decrease in total lung DNA and the total lung DNA to dry lung weight ratio. However, the small increase in total lung protein and the protein to DNA ratio along with the morphologic changes support an altered lung growth or dysplasia rather than decreased lung growth. Decreased DNA content may occur secondary to malnutrition associated with hyperoxia as well as by a direct oxygen toxic effect (18, 30). Malnutrition, however, would be unlikely in the present study since body weights were not different from controls either during oxygen exposure or during recovery. Alteration of lung growth, therefore, occurred in the absence of generalized body growth retardation.

Chronic tissue changes were apparent from saline P-V curves which demonstrated less elastic recoil, increased lung volume, and increased compliance.

Morphologically, hyperoxia-exposed lungs were characterized by dysplastic changes in both alveolar and pulmonary vascular structures. Alveolar size and shape were extremely irregular. Alveolar septa appeared foreshortened or absent resulting in enlarged air spaces. Emphysema-like changes were reflected in lung morphometrics by an increased volume proportion of parenchyma, increased mean linear intercept, decreased alveolar number, and decreased internal lung surface area. Similar lung histology was reported by both Wilson *et al.* (20) and Bucher and Roberts (18).

Burri (21) has shown that rapid alveolar growth takes place in the normal rat between 4 and 13 days of age. The present investigation suggests that hyperoxia exposure during this period results in acute inhibition of alveolar development, specifically the inhibition of secondary septation. Following hyperoxia ex-

posure, sufficient catch-up growth fails to occur and total alveolar number in the mature animal remains reduced. The finding of reduced alveolar number in the adult rat following neonatal hyperoxia exposure is similar to the effects of prolonged hyperoxia exposure in young rats (20, 31). Roberts *et al.* (19) exposed newborn rats to more than 0.95 inspired oxygen concentrations but for only for the first 6 days postnatally (19). In their study morphologic changes were attenuated by 2 wk of recovery. We assume that both timing and duration of neonatal hyperoxia exposure may be critical events determining alveolar number and morphology in the adult.

Pulmonary vascular changes associated with hyperoxia exposure include a decreased diameter of large pulmonary vessels and a striking decrease in the number of small pulmonary arterial vessels. Hyperoxia is known to inhibit vascular endothelial growth *in vitro* (15). We speculate that exposure to hyperoxia during pulmonary growth impairs branching or extension of pulmonary vessels and decreases the vascular density of the mature lung.

Reduced arterial number was also reported by Roberts *et al.* (19) and Wilson *et al.* (20). The short-term follow-up data provided by Roberts *et al.* (19) indicate that catch-up arterial growth may occur following hyperoxia exposure. The present study and the data of Wilson *et al.* (20), however, indicate that the arterial number remains reduced when evaluated in the adult rat. These differing results suggest that following the hyperoxic insult there is a tendency toward vascular recovery, but the adaptation is incomplete.

It could be hypothesized that impaired pulmonary vascularization inhibits the normal process of alveolar septal formation thus linking the two events. However, Myrick and Reid (25) have shown that septal formation precedes vascularization in the developing lung. The normal process of vascular and alveolar development appeared to be simultaneously affected by the hyperoxia. This is supported by the finding that the ratio of small pulmonary vessels per 100 alveoli was unchanged in the present study as well as the study of Wilson *et al.* (20). However, Wilson *et al.* (20) also demonstrated that more prolonged hyperoxia exposure reduces the ratio of small pulmonary vessels to alveoli suggesting that a vascular obliterative process may take place as well as vascular hypoplasia. Further dose response studies could better define the pathogenesis of these morphologic changes. It will be important to determine whether alveolar septation can be influenced independent of vascular changes.

Cardiovascular sequelae of neonatal hyperoxia exposure included the development of pulmonary hypertension and right ventricular hypertrophy. Percutaneous needle puncture of the right ventricle proved to be a useful, rapid, and reproducible method to assess maximum pulmonary arterial pressure (32, 33).

Wilson *et al.* (20) have suggested that the chronically exposed rat may adapt to an elevated inspired oxygen concentration. Return to normoxia might then stimulate pulmonary vasoconstriction similar to that seen in hypoxia-induced pulmonary hypertension. We found that oxygen saturation measurements in a group of rats following weaning from hyperoxia were similar to controls within 90 h. This indicates that reactive pulmonary vasoconstriction stimulated by relative hypoxemia was unlikely. We also assessed the lability of subsequent pulmonary hypertension by measuring RV pressure during oxygen administration as well as in RA in the adult rat. No differences in RV pressures were found. Finally, the presence of long standing pulmonary hypertension was suggested in oxygen-exposed rats by the presence of right ventricular hypertrophy.

Pulmonary hypertension may be anatomically related to increased muscularization of pulmonary arterioles, reduced number of pulmonary vessels, or generalized hypoplasia of the pulmonary vascular tree. In the present model pulmonary hypertension may be due to a combination of these events. Muscularization of arterioles was increased, vessel counts were reduced, and hypoplasia of the vascular tree was suggested by a 25%

decrease in the diameter of large pulmonary artery branches in hyperoxia-exposed rats. It should be noted, however, that muscularization of pulmonary arterioles was not dramatically increased as reported following chronic hypoxemia (34). The medial width/external diameter ratio of these vessels may have been increased in hyperoxia-exposed rats secondary to a decrease in external vessel diameter rather than a primary increase in muscularization.

We also observed changes in the distribution of the external oblique muscle surrounding pulmonary arteries of hyperoxia-exposed rats. In contrast to the increased thickness of the medial circular muscle, the external oblique muscle was found to be decreased or, in fact, absent. The pathogenesis of these divergent changes in the muscularization of pulmonary arterioles is not clear. Lane *et al.* (35) have speculated that pulmonary arterioles associated with oblique muscle are resistance vessels controlling pulmonary blood flow to alveoli. Vascular hypoplasia may have decreased pulmonary blood flow such that constant relaxation of resistance vessels was associated with atrophy of the oblique muscle. It is also possible that hyperoxia has a direct effect on the development of the oblique muscle, inhibiting its growth. The effect of oxygen concentration on the muscularization of pulmonary vessels needs to be explored further.

SUMMARY

The present investigation demonstrates that hyperoxia exposure limited to the newborn period is associated with morphologic and functional cardiopulmonary changes in the adult rat. Morphologic alterations include dysplastic alveolar and pulmonary vascular growth. Vascular pulmonary dysplasia was associated with chronic functional complications including pulmonary hypertension and increased pulmonary compliance.

The adult rat model of the toxic effects of neonatal hyperoxia exposure may be useful for future investigation of antioxidant effectiveness. Further investigation is also necessary to establish the risk of similar pulmonary abnormalities in human neonates exposed to elevated inspired oxygen concentration during comparable critical periods of lung growth and development.

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